A method for separating and purifying a nucleic acid

Technical Field

The present invention relates to a method of separating and purifying a nucleic acid. More specifically, the present invention relates to a method of separating and purifying a nucleic acid from a sample containing nucleic acids by using a membrane which has certain membrane thickness.

Background Art

The nucleic acid is used in various fields in various forms. For example, in the field of recombinant nucleic acid technology, the nucleic acid is required to be used in the form of a probe, a genomic nucleic acid, and a plasmid nucleic acid.

In diagnostic field, the nucleic acid is also used in various methods. For example, a nucleic acid probe is normally used in detection and diagnosis of a human pathogen. Similarly, the nucleic acid is used in detection of genetic disorders. The nucleic acid is also used in detection of a food contamination substance. In addition, the nucleic acid is normally used in positioning, identification and isolation of an interesting nucleic acid by various reasons such as preparation of a gene map, cloning and expression of recombinant.

In many cases, the nucleic acid can be obtained in a very small amount, and a complicated and time-consuming operation is required for isolation and purification. This frequently time-consuming and complicated operation is easy to cause a loss of the nucleic acid. In purification of the nucleic acid obtained from serum, urine and bacterial culture, risks such as occurrence of contamination and pseudopositive result are added.

One of well known purification methods is exemplified by purification by adsorption of the nucleic acid to the surface of silicon dioxide, silica polymer or magnesium silicate followed by operations such as washing and desorbing (Japanese Examined Patent Application Publication No. 1995-51065.) This

method is excellent in separation performance, however, there are problems that (1) it is difficult to industrially produce the adsorption medium of a comparable performance in a large scale, (2) the handling of the medium is inconvenient, and (3) it is difficult to process the medium in various shapes.

Summary of the Invention

An object of the present invention is to provide a method for separating and purifying a nucleic acid by adsorbing the nucleic acid in a test sample to a surface of a membrane and desorbing the nucleic acid by washing and the like. Another object of the present invention is to provide a method for separating and purifying the nucleic acid by using a membrane which is excellent in separation performance and washing efficiency, can be easily processed, and can be mass-produced for those having substantially the same separation performance.

The present inventors intensively studied to solve the above described objects. As a result, they have found that, in a method for separating and purifying a nucleic acid comprising steps of adsorbing and desorbing the nucleic acid to and from a membrane, a nucleic acid can be separated from a sample containing nucleic acids by using a membrane which has certain membrane thickness and also using an unit for separation and purification of nucleic acid which contains the membrane in a container having two openings. The invention has been completed on the basis of these findings.

According to the present invention, there is provided a method for separating and purifying a nucleic acid, comprising a step of:

adsorbing and desorbing a nucleic acid to and from a membrane of an organic macromolecule which has a membrane thickness of 10μm to 500μm.

Preferably, the organic macromolecule is an organic macromolecule having a hydroxyl group on surface thereof.

Preferably, the organic macromolecule is surface-saponified acetylcellulose.

Preferably, the organic macromolecule is surface-saponified triacetylcellulose.

Preferably, the surface-saponification rate of acetylcellulose is 5% or higher, and is more preferably 10% or higher.

Preferably, the acetylcellulose is a porous film or a non-porous film.

Preferably, the nucleic acid in a sample solution is adsorbed to and desorbed from the membrane of organic macromolecule which has a membrane thickness of 10 μm to 500 μm.

Preferably, the sample solution is a solution prepared by adding a water-soluble organic solvent to a solution obtained by treating a cell- or virus-containing test sample with a nucleic acid-solubilizing reagent.

Preferably, the nucleic acid-solubilizing reagent is a guanidine salt, a surfactant and a proteolytic enzyme.

Preferably, the method of the present invention comprises steps of:

adsorbing the nucleic acid to a membrane of the organic macromolecule;

washing the membrane using a nucleic acid-washing buffer; and

desorbing the nucleic acid adsorbed to the membrane by using a liquid capable of desorbing the nucleic acid adsorbed to the membrane.

Preferably, the nucleic acid-washing buffer is a solution containing 20 to 100 % by weight of methanol, ethanol, isopropanol or n-propanol.

Preferably, the liquid capable of desorbing the nucleic acid adsorbed to the membrane is a solution having a salt concentration of 0.5 M or lower.

Preferably, adsorption and desorption of the nucleic acid is carried out by using an unit for separation and purification of nucleic acid in which a container having at least two openings contains a membrane of the organic macromolecule which has a

membrane thickness of 10μm to 500μm.

More preferably, adsorption and desorption of the nucleic acid is carried out by using an unit for separation and purification of nucleic acid which comprises (a) a membrane of the organic macromolecule which has a membrane thickness of 10 μm to 500μm, (b) a container having at least two openings and containing the membrane, and (c) a pressure difference-generating apparatus connected to one opening of the container.

Preferably, the method according to the present invention may be carried out by the following steps of:

(a) preparing a sample solution containing a nucleic acid by using a test sample and inserting one opening of an unit for separation and purification of nucleic acid into said sample solution containing the nucleic acid;

(b) sucking the sample solution containing the nucleic acid by making an inside of the container in a reduced pressure condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and contacting the sample solution to a membrane of the organic macromolecule which has a membrane thickness of 10μm to 500μm;

(c) making the inside of the container in a pressurized condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and discharging the sample solution containing the sucked nucleic acid to an outside of the container;

(d) inserting one opening of the unit for separation and purification of nucleic acid into the nucleic acid-washing buffer;

(e) sucking the nucleic acid-washing buffer by making the inside of the container in the reduced pressure condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and contacting the nucleic acid-washing buffer

to the membrane;

(f) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and discharging the sucked nucleic acid-washing buffer to the outside of the container;

(g) inserting one opening of the unit for separation and purification of nucleic acid into the liquid capable of desorbing the nucleic acid adsorbed to the membrane;

(h) making the inside of the container in the reduced pressure condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and sucking the liquid capable of desorbing the nucleic acid adsorbed to the membrane to contact the liquid to the membrane; and

(i) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and discharging the liquid capable of desorbing the nucleic acid adsorbed to the membrane to the outside of the container.

Alternatively, the method according to the present invention may be carried out by steps of:

(a) preparing a sample solution containing the nucleic acid using a test sample and injecting said sample solution containing the nucleic acid into one opening of the unit for separation and purification of nucleic acid;

(b) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to said one opening of the unit for separation and purification of nucleic acid, and discharging the injected sample solution containing the nucleic acid from the other opening to contact the sample solution to a membrane of the organic macromolecule which has a membrane thickness of 10μm to 500μm;

(c) injecting the nucleic acid-washing buffer into said one opening of the unit for separation and purification of nucleic acid;

(d) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to said one opening of the unit for separation and purification of nucleic acid, and discharging the injected nucleic acid-washing buffer from said other opening to contact the nucleic acid-washing buffer to the membrane;

(e) injecting the liquid capable of desorbing the nucleic acid adsorbed to the membrane into said one opening of the unit for separation and purification of nucleic acid; and

(f) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to said one opening of the unit for separation and purification of nucleic acid, and discharging the liquid capable of desorbing the injected nucleic acid from said other opening, so as to desorb the nucleic acid adsorbed to the membrane and discharge the nucleic acid to the outside of the container.

Brief Description of the Drawings

Fig. 1 shows a conceptual diagram of a unit for separation and purification of nucleic acid according to the present invention;

Fig. 2 is an example of the unit for separation and purification of nucleic acid according to the present invention, wherein the pressure difference-generating apparatus to be connected to the opening 21 is not illustrated. In Fig. 2, 1 denotes a container, 10 denotes a main body, 101 denotes an opening, 102 denotes a bottom face, 103 denotes a frame, 104 denotes a wall, 105 denotes a step, 121 denotes a space, 122 denotes a space, 123 denotes a space, 13 denotes a pressing member, 131 denotes a hole, 132 denotes a projection, 20 denotes a lid, 21 denotes an opening, and 30 denotes a membrane;

Fig. 3 is a schematic diagram of a cartridge for purification of nucleic acid used in the Example; and

Fig. 4 shows the result of quantification of a collected amount of the nucleic acid which was separated and purified according to the method of the present invention and the comparative method.

Detailed Description of the Invention

Embodiments of the present invention will be explained below.

The method for separating and purifying a nucleic acid according to the present invention relates to a method of separating and purifying a nucleic acid from a sample containing nucleic acids, which is characterized in that the method comprises steps of adsorbing and desorbing the nucleic acid to and from a membrane of an organic macromolecule which has a membrane thickness of 10μm to 500μm.

The term "nucleic acid" in the invention may be a single strand or double strand, and has no limitation of a molecular weight.

The sample containing nucleic acids may be a sample containing single nucleic acid or may be a sample containing different several types of nucleic acids. The length of the nucleic acids is not particularly limited, and nucleic acids having any length ranging from several bp's to several Mbp's can be used. In view of handling, the length of nucleic acids is generally from several bp's to several hundred kbp's.

In the present invention, adsorption and desorption of the nucleic acid is carried out by using a membrane which has a membrane of the organic macromolecule which has a membrane thickness of 10 μ m to 500 μ m. Preferably, an organic macromolecule having a hydroxyl group on surface thereof can be used.

As the organic macromolecule having a hydroxyl group on surface thereof, surface-saponified acetyl cellulose is preferable. Acetyl cellulose may be any one of monoacetyl

cellulose, diacetyl cellulose and triacetyl cellulose. Particularly, triacetyl cellulose is preferable. In the present invention, it is preferable to use surface-saponified acetyl cellulose as the membrane. The surface saponification means that only surface to which a saponifying agent (e. g., NaOH) contacts, is saponified. In the present invention, it is preferable that a structural body of the membrane is kept as acetyl cellulose and only the surface of the membrane is saponified. In this way, an amount of hydroxyl groups (density) on the surface of the membrane can be controlled according to degree of surface saponification treatment (surface saponification degree).

In order to increase surface area of the organic macromolecule having a hydroxyl group on the surface, it is preferable to form the organic macromolecule having a hydroxyl group on the surface into a membrane. Further, acetyl cellulose may be a porous membrane or a non-porous membrane. However, the porous membrane is more preferable. In the case where the membrane is a porous membrane, it is preferable that the structural body of the membrane remains as acetyl cellulose and only the surface of the structural body is saponified. In this way, on the basis of the degree of surface saponification treatment (surface saponification degree) x the pore size, spatial amount of hydroxyl groups (density) can be controlled. Meanwhile, the structural body of the membrane is composed of acetyl cellulose and thus, a rigid membrane can be obtained. Here, that the surface of acetyl cellulose is saponified and hydroxyl groups are introduced to the surface only, means that the structural body is kept as acetyl cellulose and the surface is converted to cellulose. When cellulose is used as a raw material, since cellulose cannot be used in liquid, the porous membrane and a flat membrane can not be manufactured industrially.

For example, the membrane of triacetyl cellulose is marketed as a commercial name TAC base from Fuji Photo Film K.K. As the porous membrane of triacetyl cellulose, there are

Microfilter FM500 (Fuji Photo Film K.K.) and Microfilter FR250 (Fuji Photo Film K.K.; triacetyl cellulose porous membrane having a surface saponification rate of 100%).

In order to increase efficiency of separation of nucleic acid, it is preferred to increase the number of hydroxyl groups. For example, in the case of acetyl cellulose such as triacetyl cellulose, about 5% or higher of the rate of surface saponification is preferable, and 10% or higher is more preferable. Namely, the surface saponification rate of acetyl cellulose is preferably 5 to 100%, more prefarably 10 to 100%, further more prefarably 20 to 100%, further more prefarably 30 to 100%, and particularly preferably 40 to 100%.

For the surface saponification of acetyl cellulose, an object to be surface-saponified is dipped in an aqueous solution of sodium hydroxide. In order to change the surface saponification rate, the concentration of sodium hydroxide can be changed. The surface saponification rate is determined by quantifying a remaining acetyl group by NMR.

The membrane used in the present invention has a membrane thickness of 10 μm to 500 μm. When the membrane thickness is less than 10 μm, the strength of the membrane becomes weak and the physical structure cannot be maintained, which is not preferred. When the membrane thickness is more than 500 μm, the compatibility to a system for separation and purification of nucleic acid using a pressuring process (i.e., a process without centrifugation) becomes worse, which is also not preferred. The lower limit of the membrane thickness is 10 μm or more, preferably 20 μm or more, further preferably 30 μm or more, particularly preferably 40 μm or more. The upper limit of the membrane thickness is 500 μm or less, preferably 400 μm or less, further preferably 300 μm or less, further preferably 200 μm or less, particularly preferably 100 μm or less. The preferred range of the membrane thickness is for example 10 to 400 μm, more preferably 20 to 300 μm, further preferably 30 to 200 μm, and particularly preferably 40 to 100 μm.

The present invention has been completed based on the finding that the membrane thickness can be decreased to 10 to 500 μm by using a membrane of an organic macromolecule having a hydroxyl group on surface thereof such as surface-saponified triacetylcellulose, while the ability of adsorbing nucleic acid is fully retained. In the use of such a membrane having a relatively thin membrane thickness, there is an advantage that the use of such a membrane is suitable for a pressuring process (a process without centrifugation) which is convenient for automation of separation and purification operation of nucleic acids. For example, when a glass filter is used as a membrane to which a nucleic acid is adsorbed, it is difficult to use a membrane having a membrane thickness of 10 to 500 μm since the structure cannot be retained physically in such a thickness.

In the method for separating and purifying a nucleic acid according to the present invention, adsorption and desorption of the nucleic acid can be preferably conducted by using an unit for separation and purification of nucleic acid in which a container having at least two openings contains a membrane of the organic macromolecule which has a membrane thickness of 10 μm to 500 μm.

Further preferably, adsorption and desorption of the nucleic acid can be conducted by using an unit for separation and purification of nucleic acid comprising (a) a membrane of an organic macromolecule which has a membrane thickness of 10 μm to 500 μm, (b) a container having at least two openings and containing the membrane, and (c) a pressure difference-generating apparatus connected to one opening of the container.

In this case, a first embodiment of the method for separating and purifying a nucleic acid according to the invention can comprise the following steps of:

(a) preparing a sample solution containing a nucleic acid by using a test sample and inserting one opening of an unit for separation and purification of nucleic acid into said sample solution containing the nucleic acid;

(b) sucking the sample solution containing the nucleic acid by making an inside of the container in a reduced pressure condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and contacting the sample solution to a membrane of the organic macromolecule which has a membrane thickness of 10μm to 500μm;

(c) making the inside of the container in a pressurized condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and discharging the sample solution containing the sucked nucleic acid to an outside of the container;

(d) inserting one opening of the unit for separation and purification of nucleic acid into the nucleic acid-washing buffer;

(e) sucking the nucleic acid-washing buffer by making the inside of the container in the reduced pressure condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and contacting the nucleic acid-washing buffer to the membrane;

(f) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and discharging the sucked nucleic acid-washing buffer to the outside of the container;

(g) inserting one opening of the unit for separation and purification of nucleic acid into the liquid capable of desorbing the nucleic acid adsorbed to the membrane;

(h) making the inside of the container in the reduced pressure condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and sucking the liquid capable of desorbing the nucleic acid adsorbed to the

membrane to contact the liquid to the membrane; and

(i) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to the other opening of the unit for separation and purification of nucleic acid, and discharging the liquid capable of desorbing the nucleic acid adsorbed to the membrane to the outside of the container.

A second embodiment of the method for separating and purifying a nucleic acid according to the invention can comprise the following steps of:

(a) preparing a sample solution containing the nucleic acid using a test sample and injecting said sample solution containing the nucleic acid into one opening of the unit for separation and purification of nucleic acid;

(b) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to said one opening of the unit for separation and purification of nucleic acid, and discharging the injected sample solution containing the nucleic acid from the other opening to contact the sample solution to a membrane of the organic macromolecule which has a membrane thickness of 10 μm to 500 μm;

(c) injecting the nucleic acid-washing buffer into said one opening of the unit for separation and purification of nucleic acid;

(d) making the inside of the container in the pressurized condition by using the pressure difference-generating apparatus connected to said one opening of the unit for separation and purification of nucleic acid, and discharging the injected nucleic acid-washing buffer from said other opening to contact the nucleic acid-washing buffer to the membrane;

(e) injecting the liquid capable of desorbing the nucleic acid adsorbed to the membrane into said one opening of the unit for separation and purification of nucleic acid; and

(f) making the inside of the container in the pressurized

condition by using the pressure difference-generating apparatus connected to said one opening of the unit for separation and purification of nucleic acid, and discharging the liquid capable of desorbing the injected nucleic acid from said other opening, so as to desorb the nucleic acid adsorbed to the membrane and discharge the nucleic acid to the outside of the container.

The method for separating and purifying a nucleic acid by using a membrane of an organic macromolecule which has a membrane thickness of 10 to 500 μm will be described in detail below. Preferably in the present invention, the nucleic acid in the sample solution is adsorbed to a membrane of an organic macromolecule which has a membrane thickness of 10 to 500 μm by contacting the sample solution containing the nucleic acid to the membrane, and then the nucleic acid adsorbed to the membrane is desorbed from the membrane by using a suitable solution described below. More preferably, the sample solution containing the nucleic acid is a solution which is obtained by adding a water-soluble organic solvent to a solution obtained by treating a cell- or virus-containing test sample with a solution capable of solubilizing a cell membrane and a nuclear membrane to disperse the nucleic acid into the solution.

The sample solution containing the nucleic acid which can be used in the present invention is not limited, but for example, in diagnostic fields, the subject solutions are the body fluid such as whole blood, serum, plasma, urine, stool, sperm and saliva which were collected as a test sample, or solutions prepared from biological materials such as plant (or a portion thereof) and animal (or a portion thereof), or their dissolved matters and homogenates.

First, these test samples are treated with an aqueous solution containing a reagent capably of lysing the cell membrane and solubilizing the nucleic acid. By this treatment, the cell membrane and the nuclear membrane are lysed, and the nucleic acid is dispersed into the aqueous solution.

For lysing the cell membrane and solubilizing the nucleic

acid, for example, when the subject sample is whole blood, necessary steps are (1) removing erythrocytes, (2) removing various proteins, and (3) lysing leukocytes and lysing the nuclear membrane. (1) Removing erythrocytes and (2) removing various proteins are required to prevent non-specific adsorption to the membrane and clogging of the porous membrane, and (3) lysing leukocytes and lysing the nuclear membrane is required to solubilize the nucleic acid which is an object of extraction. Particularly, (3) lysing leukocytes and lysing the nuclear membrane is an important step. In the method of the present invention, it is necessary to solubilize the nucleic acid in this step. For example, by incubating the sample for 10 minutes at 60℃ under the condition in which guanidine hydrochloride, Triton X100, and protease K (Sigma made) are added, the above-mentioned (1), (2) and (3) can be achieved simultaneously.

The reagent for solubilizing the nucleic acid which is used in the present invention is exemplified by the solution containing the guanidine salt, a surfactant and a protease.

The guanidine salt is preferably guanidine hydrochloride, but other guanidine salts (guanidine isothiocyanate and guanidine thiocyanate) can also be used. The concentration of guanidine salts in the solution is 0.5 M to 6 M, preferably 1 M to 5 M.

As the surfactant, Triton X100 can be used. Alternatively, an anionic surfactant such as SDS, sodium cholate and sodium sarcosinate, a nonionic surfactant such as Tween 20 and Megafac, and other various types of amphoteric surfactants, can also be used. In the present invention, the nonionic surfactant such as polyoxyethylene octylphenyl ether (Triton X100) is preferably used. The concentration of the surfactant in the solution is normally 0.05% by weight to 10% by weight, particularly preferably 0.1% by weight to 5% by weight.

As the protease, Protease K can be used, but other proteases can also give same effect. The protease is an enzyme

and thus, incubation is preferable. The protease is preferably used at 37℃ to 70℃, particularly preferably at 50℃ to 65℃.

An aqueous organic solvent is added to the aqueous solution in which the nucleic acid is dispersed, to contact the nucleic acid to the organic macromolecule. By this operation, the nucleic acid in the sample solution is adsorbed to the organic macromolecule. In order to adsorb the nucleic acid which was solubilized by the operation as described hereinabove to the membrane of the organic macromolecule, it is necessary that an aqueous organic solvent is mixed with the solubilized nucleic acid mixture solution, and a salt is present in the obtained nucleic acid mixture solution.

By breaking a hydrating structure of a water molecule present around the nucleic acid, the nucleic acid is solubilized in an unstable state. It is presumed that when the nucleic acid in such state is contacted to the membrane of the organic macromolecule, a polar group on the surface of the nucleic acid interacts to the polar group on the surface of the membrane and the nucleic acid is adsorbed to the surface of the membrane. In the method of the present invention, the state of the nucleic acid can become unstable by mixing the aqueous organic solvent with the solubilized nucleic acid mixture solution and by the presence of the salt in the obtained mixture solution of the nucleic acid.

The aqueous organic solvent used herein is exemplified by ethanol, isopropanol or propanol. Among them, ethanol is preferable. The concentration of the aqueous organic solvent is preferably 5% by weight to 90% by weight, and more preferably 20% by weight to 60% by weight. It is particularly preferable to make the concentration of ethanol to be added as high as possible in a degree in which no coagulant occurs.

As the salt present in the obtained mixture solution of the nucleic acid, various chaotropic substances (guanidium salt, sodium iodide, and sodium perchlorate), sodium chloride, potassium chloride, ammonium chloride, sodium bromide, potassium bromide, calcium bromide, ammonium bromide and the

like are pref rable. Particularly, guanidium salt has both effects of lysis of cell membrane and solubilization of the nucleic acid, and therefore is particularly preferable.

Subsequently, the organic macromolecule to which the nucleic acid is adsorbed, is contacted to the nucleic acid-washing buffer solution. This buffer solution has a function of washing out impurities in the sample solution which are adsorbed to the organic macromolecule together with the nucleic acid. Consequently, the solution should have a composition having no ability of desorbing the nucleic acid from the organic macromolecule and an ability of desorbing the impurities. The nucleic acid-washing buffer solution is an aqueous solution comprising a main agent, a buffer agent and when required, a surfactant. The main agent is exemplified by an about 10 to 100% by weight (preferably about 20 to 100% by weight and more preferably about 40 to 80% by weight) aqueous solution of methanol, ethanol, isopropanol, n-propanol, butanol, acetone and the like. The buffer agent and the surfactant are exemplified by the previously described buffer agents and surfactants. Among them, a solution containing ethanol, Tris and Triton X100 is preferable. The preferable concentrations of Tris and Triton X100 are 10 to 100 mM and 0.1 to 10% by weight, respectively.

Then, to the solution capable of desorbing the nucleic acid adsorbed to the organic macromolecule, is contacted the washed organic macromolecule as described above. This solution contains the target nucleic acid and hence, is collected and subjected to amplification of the nucleic acid by following operation, e.g., PCR (polymerase chain reaction). It is preferable that the solution capable of desorbing the nucleic acid has a low salt concentration and particularly preferably, the solution of 0.5 M or lower salt concentration is used. For this solution, purified distilled water, TE buffer and the like can be used.

The unit for separation and purification of nucleic acid which is used in the invention is an unit for separation and

purification of nucleic acid wherein the membrane of the organic macromolecule which has a membrane thickness of 10μm to 500 μm is contained in the container having at least two openings.

The material of the container is not particularly limited, so long as the organic macromolecule is contained therein and at least two openings can be provided. In view of easiness of manufacturing, a plastic is preferable. For example, clear or opaque resins such as polystyrene, polymethacrylate ester, polyethylene, polypropylene, polyester, nylon, or polycarbonate are preferably used.

Fig. 1 shows a conceptual diagram of the container. Basically, the container has a section for containing the membrane, and the membrane is contained said containing section. The membrane does not move out of the containing section at the time of sucking and discharging the sample solution and the like. A pressure difference-generating apparatus, e. g., a syringe, is connected to the opening. For this purpose, it is preferable that the container is initially divided into two sections, and after the membrane is contained, these portions are integrated. In addition, in order to prevent that the membrane moves out of the containing section, a mesh made of the material which does not contaminate DNA, can be placed on the top and the bottom of the membrane.

There is no limitation on the shape of the membrane of the organic macromolecule which is contained in the container as described above. The shape may be any shape such as discoid, squared, rectangular or ellipsoid; and in the membrane, cylindrical, roll, or beads coated with the organic macromolecule having a hydroxyl group on the surface. In view of manufacturing suitability, the shape having symmetric property such as discoid, squared, cylindrical and roll, and beads are preferable.

The one opening of the container described above is inserted into the sample solution containing the nucleic acid, and the sample solution is contacted to the organic macromolecule by sucking from the other opening. The sample

solution is discharged, and then the nucleic acid-washing buffer solution is sucked and discharged. Then, the solution capable of desorbing the nucleic acid adsorbed to the organic macromolecule is sucked and discharged. This discharged solution is collected to obtain the target nucleic acid.

Alternatively, by dipping the organic macromolecule in the sample solution containing the nucleic acid, the nucleic acid-washing buffer solution, and the solution capable of desorbing the nucleic acid adsorbed to the organic macromolecule, in this order, the target nucleic acid can be obtained.

The unit for separation and purification of nucleic acid used in the invention preferably comprises (a) a membrane of the organic macromolecule which has a membrane thickness of 10 μm to 500 μm, (b) a container containing the membrane and having at least two openings, and (c) a pressure difference-generating apparatus connected to one opening of the container. The unit for separation and purification of nucleic acid will be described below.

The container is normally made in a divided form of a main body which contains the membrane of the organic macromolecule, and a lid, wherein each has at least one opening. The one is used as an inlet and an outlet of the sample solution containing the nucleic acid, the nucleic acid-washing buffer solution, a liquid capable of desorbing the nucleic acid adsorbed to the membrane (hereinafter referred to as "sample solution and the like"); and the other is connected to the pressure difference-generating apparatus capable of making the inside of the container in a reduced pressure or pressurized state. There is no limitation of the shape of the main body. In order to make manufacture easy and also make entire diffusion of the sample solution on the membrane easy, it is preferable that the section is a circular shape. In order to prevent a cutting wastage of the membrane, it is also preferable that the section is a squared shape.

It is necessary to connect the lid to the main body so

as to make the inside of the container in the reduced pressur state or the pressurized state by using the pressure difference-generating apparatus. If this state is accomplished, the method of connection can be selected freely. For example, use of an adhesive, screwing, fitting, securing, and fusing by ultrasonic heating, are exemplified.

An internal volume of the container is determined only by an amount of the sample solution to be treated. Normally, it is expressed by the volume of the membrane to be contained. It is preferable to use a size suitable for containing 1 to 6 sheets of the membrane having about 1 mm or smaller (e. g., around 50 to 500 μm) thickness and about 2 mm to 20 mm diameter.

It is preferable to make an end of the membrane to contact closely to an inner wall face of the container to prevent the sample solution and the like from being passed.

The bottom surface of the membrane that is located oppositely to the opening used as the inlet of the sample solution and the like, is not closely contacted to the inner wall of the container, and a space is provided. Thereby, a structure suitable for achieving diffusion of the sample solution and the like evenly as possible on the entire surface of the membrane, can be formed.

It is preferable to provide a member having a hole in generally a center thereof on the membrane located oppositely to the other opening, i. e., the opening connected to the pressure difference-generating apparatus. This member pushes the membrane, and has an efficient effect of discharging the sample solution and the like. This member has preferably a shape having a slope such as a funnel or a cup in such a way that the liquid is collected in the center hole. The size of this hole, an angle of the slope, and the thickness of the member can be properly determined by those skilled in the art in consideration of the amount of the sample solution and the like to be treated and the size of the container for containing the membrane. Between this member and the opening, a space is preferably provided to store the overflowed sample solution and

the like and prevent the sample solution from being sucked into the pressure difference-generating apparatus. The volume of this hole can be properly chosen by those skilled in the art. In order to collect efficiently the nucleic acid, it is preferable to suck the sample solution containing the nucleic acid in an amount which is sufficient for dipping a whole of the membrane.

In order to prevent the sample solution and the like from being concentrated only beneath the opening through which sucking is carried out and to allow the sample solution and the like to be passed through the membrane, a space is preferably provided between the membrane and this member. For this purpose, it is preferable to provide a plurality of projection from the member to the membrane. The size and number of the projection can be properly chosen by those skilled in the art. It is preferable to make an opening area of the membrane as large as possible while keeping the space.

When the container has 3 or more openings, in order to make possible sucking and discharging the liquid by pressure-reducing and pressuring operations, it is needless to say that an excess opening should be closed temporarily.

The pressure difference-generating apparatus reduces at first the pressure of the inside of the container which contains the membrane, so as to suck the sample solution containing the nucleic acid. The pressure difference-generating apparatus is exemplified by the syringe, pipetter, or a pump capable of sucking and pressurizing such as a peristaltic pump. Among them, the syringe is suitable for manual operation and the pump is suitable for automatic operation. The pipetter has an advantage of one hand operation. Preferably, the pressure difference-generating apparatus is releasably connected to the one opening of the container.

Next, the purification method of the nucleic acid using the unit for separation and purification of nucleic acid as described above, will be described. First of all, in the sample solution containing the nucleic acid is inserted one opening

of the unit for separation and purification of nucleic acid as described above. Then, by using the pressure difference-generating apparatus connected to the other opening, the pressure of the inside of the purifying unit is reduced to suck the sample solution into the container. By this operation, the sample solution is contacted to the membrane so as to adsorb the nucleic acid present in the sample solution to the membrane. At this time, it is preferable to suck the sample solution in such an amount that the solution can be contacted to almost whole of the membrane. However, sucking of the solution in the pressure difference-generating apparatus causes contamination of the apparatus and hence, the amount is appropriately adjusted.

After an appropriate amount of the sample solution is sucked, the inside of the container of the unit is pressurized by using the pressure difference-generating apparatus, and the sucked liquid is discharged. No interval is required for this operation, and discharge may be carried out immediately after sucking.

Subsequently, the nucleic acid-washing buffer solution is sucked into the container and discharged from it by pressure-reducing and pressurizing operations as described above to wash the inside of the container. This solution has functions of washing out the sample solution left in the container and also washing out impurities in the sample solution adsorbed to the membrane together with the nucleic acid. Therefore, the solution must have a composition having functions of desorbing no nucleic acid but impurities from the membrane. The nucleic acid-washing buffer solution is an aqueous solution containing a main agent, a buffer agent and when required, a surfactant. The main agent is exemplified by about 10 to 90% (preferably about 50 to 90%) aqueous solution of methyl alcohol, ethyl alcohol, butyl alcohol, acetone and the like; and the buffer agent and the surfactant are exemplified by the previously described buffer agents and surfactants. Among them, a solution containing ethyl alcohol,

Tris and Triton X100 is preferable. The preferable concentrations of Tris and Triton X100 are 10 to 100 mM and 0.1 to 10%, respectively.

Next, a solution capable of desorbing the nucleic acid adsorbed to the membrane is introduced into the inside of the container and discharged from the container by pressure-reducing and pressurizing operations as described above. This discharged solution contains the target nucleic acid and hence, the target nucleic acid can be collected to be subjected to amplification of the nucleic acid by a following operation, e.g., PCR (polymerase chain reaction).

Fig. 2 is a sectional view of an example of the unit for separation and purification of nucleic acid according to the present invention, provided that no pressure difference-generating apparatus is illustrated. A container 1 containing the membrane comprises a main body 10 and a lid 20 and is made of clear polystyrene. The main body 10 contains saponified triacetyl cellulose membrane as a membrane 30. In addition, it has an opening 101 for sucking the sample solution and the like. A bottom face 102 extending from the opening is formed in a funnel shape, and a space 121 is formed between this and the membrane 30. In order to support the membrane 30 and hold the space 121, a frame 103 which is formed with the bottom face 102, is provided.

The main body has an inner diameter of 20.1 mm, a depth of 5.9 mm, and a length from the bottom face 102 to the opening 101 of about 70 mm. The membrane 30 which is contained has a diameter of 20.0 mm. The thickness of one sheet of the membrane is about 50 to 500 μm, and an example of the thickness is 100 μm.

In Fig. 2, a funnel-shaped pressing member 13 is provided on the top of the membrane. A hole 131 is made in a center of the pressing member 13, and a group of projections 132 are provided downward, and a space 122 is formed between this and the membrane 30. To prevent leaking of the sample solution and the like from a space between the membrane 30 and a wall 104

of the main body 10, the inner diameter of the upper portion of the wall 104 is larger than the diameter of the membrane. The periphery of the pressing member 13 is mounted on a step 105.

A lid 20 is connected to the main body 10 by ultrasonic heating. In almost central part of the lid 20 is provided an opening 21 for connecting the pressure difference-generating apparatus. Between the lid 20 and the pressing member 13 is provided a space 123 for holding the sample solution and the like which flow out from the hole 131. A volume of the space 123 is about 0.1 mL.

The present invention will be described in more detail with reference to examples. However, the present invention is not limited to these examples.

Examples

Example 1

(1) Preparation of cartridge for purification of nucleic acid

A cartridge for purification of nucleic acid having a portion which has 7 mm inner diameter and 2 mm thickness for containing a solid phase (membrane) for adsorption of a nucleic acid, was prepared by high impact polystyrene. Fig. 3 shows a structure of the prepared cartridge for purification of nucleic acid. This cartridge for purification of nucleic acid has an opening for sucking a sample, a portion for containing a nucleic acid-adsorbing solid phase (membrane), and an opening for discharging the sample. A suction pump is connected to the opening for discharging the sample to suck the sample.

(2) Preparation of solid phase for purification of nucleic acid

Fuji Microfilter FR250 (Fuji Photo Film K.K.; triacetyl cellulose porous membrane having a membrane thickness of 70 μm and a surface saponification rate of 100%) was used as a solid phase for adsorbing nucleic acid. As a comparative solid phase for adsorbing nucleic acid, Glass filter GF/D (Whatmann) (membrane thickness of 2mm) was used.

These solid phases were contained in the portion for

containing a nucleic acid-adsorbing solid phase, of the cartridge for purification of nucleic acid which was prepared in the above (1).

(3) Preparation of adsorption buffer solution and washing buffer solution for purification of nucleic acid

An adsorption buffer solution and a washing buffer solution for purification of nucleic acid, the compositions of which are shown in Table 1, were prepared.

Table 1

[adsorption buffer]	
Guanidine hydrochloride (Life Technology made)	382 g
Tris (Life Technology made)	12.1 g
Triton X100 (ICN made)	10 g
Distilled water	1000 mL
[washing buffer]	
10 mM Tris-HCl 65% ethanol	

(4) Operation of nucleic acid purification

A culture solution of cancerous human myelocytes (HL60) was prepared. To 200 μl of this culture solution were added 200 μL of the adsorption buffer and 20 μl of protease K (SIGMA), and the mixture was incubated for 10 minutes at 60℃. After incubation, 200 μL of ethanol was added, and the mixture was stirred. After stirring, the solution was sucked and discharged by using cartridges for purification of the nucleic acid having the solid phase for purification of nucleic acid which were prepared in the above (1) and (2).

Moreover, impurities on the cartridge and the adsorbing solid phase were washed out by sucking and discharging 500 μL of the washing buffer.

Finally, 200 μL of distilled water was sucked to collect this liquid.

(5) Quantification of the amount of collected nucleic acid

The above mentioned experiment was r peated 9 times using the solid phase for adsorbing nucleic acid for the present invention. The above mentioned experiment was repeated 10 times using the solid phase for adsorbing nucleic acid for the comparative example. By measuring an optical absorption of each of the collected liquid at 260 nm, an amount of the collected DNAs was quantified. The results are shown in Table 2 and Fig. 4.

Table 2

name	230	260	280	260/280	ng/μl	vol.(μl)	(μg)	
Present Invention	0.459	0.887	0.450	1.971	89	200	17.7	
	0.536	1.087	0.544	1.998	109	200	21.7	
	0.451	0.941	0.465	2.024	94	200	18.8	
	0.404	0.873	0.427	2.044	87	200	17.5	
	0.432	0.913	0.452	2.020	91	200	18.3	
	0.389	0.818	0.405	2.020	82	200	16.4	
	0.473	0.959	0.477	2.010	96	200	19.2	18.5
	0.442	0.923	0.459	2.011	92	200	18.5	SD=1.5
	0.436	0.920	0.454	2.026	92	200	18.4	CV=8%
Comparative	0.350	0.648	0.334	1.940	65	200	13.0	
	0.177	0.274	0.154	1.779	27	200	5.5	
	0.148	0.223	0.125	1.784	22	200	4.5	
	0.140	0.160	0.087	1.839	16	200	3.2	
	0.449	0.862	0.437	1.973	86	200	17.2	
	0.327	0.558	0.287	1.944	56	200	11.2	
	0.121	0.152	0.091	1.670	15	200	3.0	
	0.205	0.350	0.184	1.902	35	200	7.0	7.3
	0.201	0.302	0.166	1.830	30	200	6.0	SD=4.9
	0.121	0.139	0.085	1.635	14	200	2.8	CV=66%

As is understood from the results of Table 2 and Fig. 4, it has been found that a nucleic acid can be recovered and purified efficiently with excellent reproducibility by using the solid phase for adsorbing nucleic acid for the present invention.

Industrial Applicability

By the method for separating and purifying a nucleic acid according to the present invention, which uses a membrane which is excellent in separation performance, good in washing efficiency, easy in processing, and is capable of mass-production of those having substantially same separation performance, a nucleic acid can be separated and purified from a sample containing nucleic acids. In addition, operations become easy by using the unit for separation and purification of nucleic acid which was described in the present specification.